REMARKS/ARGUMENTS

Claims 1-5, 8-14, 21-34, 37-43, and 50-56 are pending.

Claims 1, 9, 28-30, and 38 have been amended.

Claims 6-7, 15-20, 35-36, and 44-49 have been cancelled.

Support for the amendments is found in the claims and specification (e.g., pages 30-31 and fig. 4(a)-(b)) as originally filed.

No new matter is believed to have been added.

Claims 1, 2, 4-9, 11-14, 19, 21, 24, 25, 27-31, 33-38, 40-43, 48-50, and 56 are rejected under 35 U.S.C. 103(a) over Misako et al., JP 2003-174900, Schelper et al., *J. Bicteriol.*, 179(24):7803-7811 (1997), and Burns et al., US 6,379,929. The rejection is traversed because:

(1) the combination of the references does not describe (a) the flow channel comprising a circular flow channel comprising the regeneration region and the denaturation region, and (b) that the temperature of a regeneration region is controlled at 30-40 °C,

(3) the Schleper polymerase is not described to be used in PCR,

(4) there is no motivation of using the Schleper enzyme at 30-40 °C in the third chamber of the Misako amplification device, and

(5) combining the Burns et al., Misako et al., and Schelper et al. is technically improper.

Misako et al. describe an amplification process in which a reaction solution passes repeatedly regeneration and denaturation regions and an amplifier having a spiral-type flow channel, a heat-at-high-temperate chamber, a low temperature chamber, and a moderate temperature chamber (fig. 3). Misako et al. describe using "heating at high temperature section 5" (fig. 3), i.e., a usual PCR process utilizing a thermostable polymerase (e.g., amplifying at around 72 °C) because the chamber is set at that temperature.

Misako et al. do not use "a circular flow channel" as alleged by the Examiner. The Misako et al. channel is a spiral-type channel, i.e., a reaction solution does not pass the same circular channel reputedly (see fig. 4 a-b of the present specification for illustration) but flows along a different part of a spiral-type channel every time the reaction solution passes a renaturation and denaturation region (see fig. 3 of Misaka et al.).

Further, Misaka et al. do not describe a regeneration region chamber controlled at 30 to 40 °C, which is too low for the DNA polymerase of Misako et al. Using a low optimal temperature synthetase is advantageous because enzymes that are not used in a conventional PCR (e.g., described by Misako et al.) can be used to improve reliability of the amplification (see the paragraph bridging pages 8-9).

Also, a polymerase having a lower optimum temperature, e.g., a DNA polymerase Klenow fragment from *E.coli* having an optimum temperature of around 37 °C, cannot be used in the Misako PCR. Specifically, in a conventional solution PCR, the time-lag might occur for the enzyme having a lower optimum temperature to go into activation from the denaturing conditions. However, in combination with the flow-type PCR, a DNA polymerase having a lower optimum temperature (e.g., 30-40 °C) is ready to do its task because it is sealed in an optimum condition at the beginning of the regeneration region. Because of the lower temperature condition in which the enzyme is immobilized, the enzyme is more stable than that in the Misako PCR. Stability of the enzyme is more likely to be achieved at a lower temperature, if compared to an enzyme having an optimum temperature at around 72 °C of the Misako PCR.

Moreover, the Schleper polymerase is not described to be used in PCR. There is no disclosure in Schleper that an amplification of DNA can be accomplished reliably with the *Cenarchaeum symbiosum* polymerase. Schleper only discloses that *C. symbiosum* DNA polymerase exhibits its highest specific activity with a gapped-duplex DNA as a substrate

(abstract, fig. 4), but does not teach using the isolated enzyme for DNA amplification such as disclosed in Misako. Also the optimum temperature of the polymerization activity on the gapped-duplex DNA of the Schleper enzyme is 42°C, while its 3'-5'-*exonuclease activity* temperature is 38 °C (fig. 4, and page 7807, right col) (see claims 1-5, 7-14, and 19-27).

There is no motivation of using the Schleper enzyme at 30-40 °C in the third chamber of the Misako et al. amplification device. Indeed, one would not have used Schleper's enzyme in a regeneration chamber controlled at 30-40 °C because of the enzyme's high exonuclease activity 38 °C.

Burns et al. describe a chip-based isothermal DNA amplification (Strand Displacement Amplification ("SDA")) that requires a number of enzymes in addition to a DNA polymerase which are not necessary for the traditional PCR (col. 18, lines 39-62). In the isothermal DNA amplification, heat is not used to denature double-stranded nucleic acids because the reaction employs additional enzymes with different biological activities (col. 18, lines 39-45) and separate denaturation and regeneration regions do not exist in SDA. Also, SDA may be conducted at a temperature of 20-75 °C (col. 14, lines 29-56).

Burns et al. do not describe (a) maintaining a temperature of the regeneration region at 30-40 °C and (b) having a temperature of the denaturation region higher than that of the regeneration region (see present claim 2). The Burns et al. amplification as a whole is conducted at a constant temperature of 20-75 °C and does not require denaturation by heat (col. 14, lines 29-56; col. 16, lines 53-60; col. 73, lines 10-13 (Example 2)). In addition, a DNA polymerase in SDA must lack 5'-3' exonuclease activity (col. 18, lines 48-50).

Misako et al. process requires denaturation of dsDNA at a high temperature and the temperature of the regeneration and denaturation regions to be different. The Schleper enzyme has 5'-3' exonuclease activity (abstract, Table 2) and, therefore, cannot be used in the Burns et al. amplificaiton.

Thus, combining the isothermal DNA amplification of Burns et al. with the Misako et al. PCR and the Schleper enzyme is technically improper because the Misako et al. PCR requires heat denaturation and a different temperature during denaturation and regeneration and the Schleper enzyme has 5'-3' exonuclease activity.

Thus for the reasons set forth above, Misako, Schleper, and Burns do not make the claimed invention obvious. Applicants request that the rejection be withdrawn.

Claims 3, 10, 13, 20, 22-23, 32, 39, and 51-54 are rejected under 35 U.S.C. 103(a) over Misako et al., Schleper et al., Burns et al., and Moses, Mol. And Cellular Biol., 14(4):2767-2776 (1994).

Claims 5 and 12-14 are rejected under 35 U.S.C. 103(a) over Misako et al., Schleper et al., Burns et al., and Hideo, JP 6-30776.

Claim 8 is rejected under 35 U.S.C. 103(a) over Misako et al., Schleper et al., *J. Bicteriol.*, Burns et al., and Southgate, US 5,863,801.

Claims 26 and 55 are rejected under 35 U.S.C. 103(a) over Misako et al., Schleper et al., Burns et al., and Belford, *Biotech. Bioengineering*, 33:1047-66 (1989).

The rejection is traversed because combinations of the combination of the references do not describe or suggest that (1) the flow channel comprises a circular flow channel comprising the regeneration region and the denaturation region, and (2) the temperature of a regeneration region is controlled at 30-40 °C.

The disclosures of Misako et al., Schleper, and Burns et al. are discussed above. Moses, Hideo, Southgate, and Belfort do not cure the deficiency of Misako et al., Schleper, and Burns et al..

Moses teaches that a DNA polymerase bound to an affinity column is active (e.g., fig. 2 and page 2768, right col.).

Hideo teaches carrying an amplification reaction by transporting a reaction solution as a mobile phase through various reaction sections (fig. 1-2, abstract).

Southgate describes a device and a method for automatic isolation of a nucleic acid comprising pumps that can reverse the flow of reagents (col. 20, lines 11-25).

Belfort describes membrane bioreactors (e.g., page 1051-53).

However, Moses, Hideo, Southgate, and Belfort do not teach or suggest the flow channel comprising a circular flow channel comprising the regeneration region and the denaturation region, using a low temperature synthetase for amplification and an amplifier having a regeneration region controlled at 30-40 °C.

Thus, the cited references do not make the claimed invention obvious. Applicants request that the rejection be withdrawn.

A Notice of Allowance for all pending claims is requested.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.
Norman F. Oblon

Marina I. Miller, Ph.D.
Registration No. 59,091

Customer Number
22850

Tel: (703) 413-3000
Fax: (703) 413 -2220
(OSMMN 08/07)